



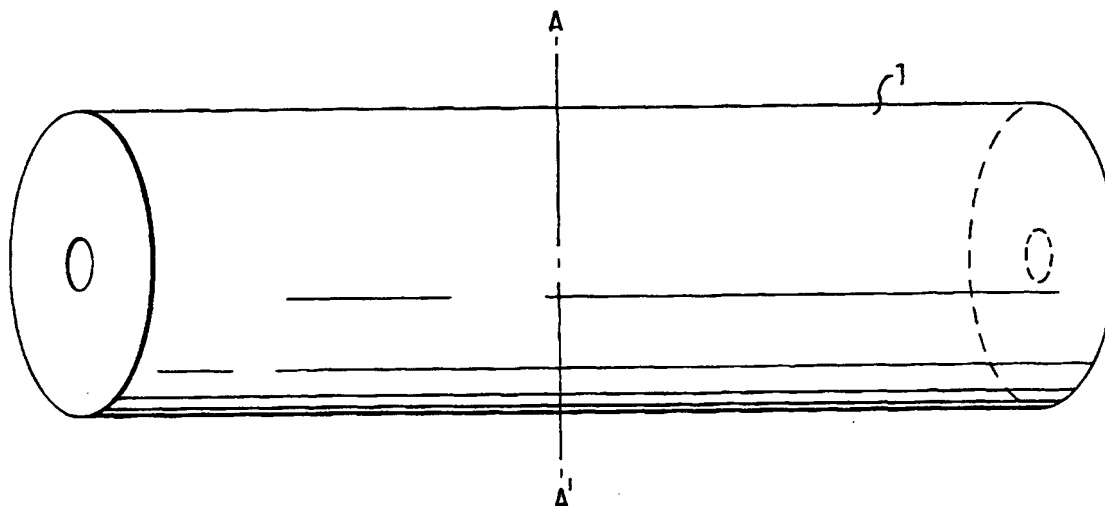
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(54) Title: APPARATUS AND METHODS FOR CULTURING BIOLOGICAL MATERIAL



(57) Abstract

A method of providing a substrate with a textured surface comprising the steps of forming a mobile surface studded with a granular material on the substrate, setting the mobile surface and removing the granular material to leave a textured surface on said substrate, and apparatus for use in culturing micro-biological material comprising a substrate produced by such a method.

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- 1 -

**APPARATUS AND METHODS FOR CULTURING
BIOLOGICAL MATERIAL**

DESCRIPTION

5

The present invention relates to vessels having textured surfaces for growing animal cells, plant cells, micro-organisms and the like ('bio-substances') and to methods for their production.

10

Many bio-substances can be propagated *in vitro* in containers such as so-called 'roller bottles', that is, cylindrical vessels, usually of glass or polystyrene, which are partially filled with a liquid medium
15 comprising the bio-substances. The bottles are arranged to be rotated slowly about their longitudinal axes, promoting aeration, while the liquid medium provides nutrients to the bio-substances as they grow on the inner surfaces of the container.

20

Limitations in the efficiency of conventional procedures have hitherto been due to the relatively small surface area provided by the smooth interior of the container, as well as the fact that such surfaces do not provide
25 optimum characteristics for the growth processes. It is an object of the present invention to eliminate or minimise these disadvantages.

- 2 -

Accordingly, in a first aspect of the invention, there is provided apparatus for use in a method of culturing micro-biological material, wherein said apparatus defines a rough or uneven growth surface arranged for contact
5 with micro-biological material being cultured. An advantage of the inventive apparatus is that its use can allow cultured material to be grown at enhanced rates.

Preferably, the growth surface is rendered rough or
10 uneven by surface features having a depth of 1mm or less and, preferably, a depth of 0.1-0.5mm. In preferred embodiments, the growth surface is pitted, or pockmarked with a plurality of craters or crater like depressions, preferably to depths of 1mm or less and more preferably
15 to depths of 0.1-0.5 mm. Preferably, the craters or crater like depressions measure less than 5, preferably less than 2, and more preferably less than 1mm across. Most preferably, the crater or crater like depressions measure less than 0.5mm across.

20

In further embodiments, the growth surface is micro-cupellated and it can be formed from an organic polymer. The polymer can be coated onto a supporting substrate and, preferably, is a silicone rubber. In an embodiment,
25 the growth surface forms an inner surface of a container for retaining biological material being cultured.

In a second aspect, the invention provides a method of

culturing a micro-biological material, comprising culturing said material using apparatus defining a growth surface, wherein said material is in contact with the growth surface and the growth surface is uneven or rough.

5 Advantageously the inventive method allows biological material to be grown at an enhanced rate.

Preferably, the apparatus employed in the second aspect of the invention is in accordance with its first aspect.

10

In a third aspect, the invention provides a method of providing a substrate with a textured surface comprising the steps of forming a mobile surface studded with a granular material on the substrate, setting the mobile

15 surface and removing the granular material to leave a textured surface on said substrate.

In a fourth aspect of the invention, there is provided an article having a textured surface that can be obtained by

20 a method according to the invention in its third aspect. Preferably, the article is a tissue or cell culture vessel, such as a roller-bottle.

In all aspects of the invention the biological material

25 preferably comprises tissue, cells or like matter, including viral particles or virions.

In the invention according to its fourth aspect, the

mobile surface is preferably tacky prior to the setting step, so as to promote adherence of the granular material. In a preferred embodiment, a (preferably solvent-less) silicone paint, designed for polymerisation
5 to form silicone rubber, is applied to the interior surface of a glass or polystyrene roller bottle. While the coating is still in liquid form, common salt particles are injected into the container, for example, under pressure, to adhere to the prepared silicone
10 coating, with any excess salt being removed again from the container. The coating is then allowed to polymerise into a solid layer at ambient or elevated temperature, depending on the type of silicone used. When hardened, the salt particles adhering to the coating are removed by
15 dissolution in water, to leave a surface-porous layer exhibiting a cratered or micro-cupellated structure, as well as the special affinity for bio-substances of silicone rubber. The container so prepared is found to result in a greatly increased yield when used for growth
20 processes in the manner described.

To promote adhesion of the silicone rubber layer within a glass or polystyrene container, a conventional adhesive, preferably a mineral spirit based primer, can
25 be first applied to the surface prior to deposition of the silicone layer.

In embodiments of the invention, it is a pre-requisite

that the growth surface should be non-toxic and inert in respect of bio-substances and liquid media. The method of creating such surfaces should not involve any materials, which if remaining on the surface as impurities, could
5 have a deleterious effect on the growth process.

In the above described method, various types of particles can be used in place of common salt, such as, for example, sugar, saltpetre and the like; however, common
10 salt is preferred as being non-toxic and inert in respect of normal media and bio-substances, as well as being soluble in water for subsequent removal. Solubility of the particles in a non-toxic solvent is an important factor in their choice.

15

Attempts have been made to use 'open-porous' silicone rubbers, such as the material 'Immobilasil' ('Immobilasil' is a registered trade mark); however, the results obtained were not as satisfactory as those achievable using the
20 various aspects of the present invention. The open-porous structure (that is, the presence of inter-connected pores within the body of the silicone rubber) in such known materials leads to difficulties in the subsequent harvesting of the biosubstances from the interstices of
25 the coating on completion of a growth cycle.

In an embodiment of the invention, surface pitting or cratering, for example, micro-cupels, can be produced in

the inner surfaces of glass or polystyrene containers per se. For this purpose, salt or other suitable particles are injected, for example, pneumatically, into the container while the latter is at elevated temperature, either during its manufacture or on subsequent heating. Upon cooling, the particles are then removed as described above, leaving the interior surface of the glass or polystyrene container with a textured, cratered or micro-cupellated structure, again resulting in enhanced efficiency of the process. In the case of polystyrene containers, prior to use, the standard procedure of plasma treatment of the inner surface can be applied to impart a negative electrical charge to the surface, as required for the growth process. Other types of particle can be used apart from salt, the latter however, being again preferred for the reasons enumerated.

It is evident that other methods lying within the ambit of the invention may also be used to derive surface porous, cratered or micro-cupellated textured surfaces of the nature described for growing of bio-substances. The invention can equally be applied to all suitable containers, such as, for example, flasks, tubes, trays, roller bottles, plastic bags and the like.

25

In applications where very large area growth surfaces are required, it is conventional to utilise containers which comprise flat plates, for example, of polystyrene, wetted

by the liquid medium, and upon which the bio-substances are made to grow. Such plates can also be provided with coatings of silicone rubber having a cratered or micro-cupellated, porous, surface structure according to the invention, in the manner described above, to result in substantially enhanced productivity.

Thus, roller bottles and other containers according to the invention can have a specially textured, cratered or micro-cupellated growth surface, usually an inner surface, suitable for use in the growth of bio-substances as aforesaid. By their nature, these surfaces are more conducive to the adhesion and growth of bio-substances, with resulting improved process efficiency. It is considered that the larger surface areas provided by such growth surfaces is a factor in their enhanced performance.

In order that the invention may be better understood, an example thereof will now be described by way of illustration only and with reference to the accompanying drawings, wherein:

Figure 1 is a schematic front perspective view of a conventional roller bottle;

Figure 2 is a schematic side cross-sectional view of the roller bottle of Figure 1 along the line AA' after

partial treatment by the inventive method;

Figure 3 is a schematic side cross-sectional view of the roller bottle of Figure 2 after treatment by the
5 inventive method has been completed; and

Figure 4 is a schematic scrap view from a point on the axis of the roller bottle showing a portion of the growth surface after treatment by the inventive method has been
10 completed.

With reference to Figures 1 to 4, a polystyrene roller bottle 1 is provided with a cratered or micro-cupellated surface according to the inventive method as follows. To
15 the interior surface of bottle 1, a coating 2 of silicone rubber primer SS 4155 (General Electric Co., Connecticut, U.S.A.) is applied by brushing or other means and allowed to dry at 70°C for 15 minutes. A coating 3 of
silicone rubber paint RTV 118 (General Electric Co.,
20 Connecticut, U.S.A.), approximately 0.05 to 0.2mm in thickness, is applied to primer coating 2 and left to stand for 1 minute. A substantial quantity of common salt 4 is then introduced into bottle 1 until
approximately 1/3 full and the latter shaken, so that
25 salt 4 adheres to all of the paint coating 3. The excess salt 4 is removed and coating 3 allowed to cure at 70°C for 30 mins. Bottle 1 is then filled with boiling water and allowed to stand for 10 minutes before the water is

removed. This last step is repeated once before bottle 1 is rinsed with isopropyl alcohol and left to dry at 70°C for 30 mins. The resultant roller bottle 1 has a textured interior surface 5 provided by craters or micro-cupels 6 inset in silicone rubber layer 4.

The roller bottle 1 can then be inoculated with a biological material, for example cells taken from a commercially available cell line such as Madin Darby
10 Canine Kidney, suspended in an appropriate commercially available nutrient medium, such as Glucose Minimum Essential Medium containing 5% foetal calf serum, selected for its suitability for growing the chosen cells. The roller bottle can then be placed upon
15 conventional equipment designed to gently roll the bottle under conditions conducive to growing cells from the selected line. For example, a common culturing temperature is 37°C. After an appropriate culturing period, the growing cells can be harvested from the
20 roller bottle using conventional techniques, such as trypsinisation or mechanical scraping.

CLAIMS

1. Apparatus for use in a method of culturing micro-
5 biological material, wherein said apparatus defines a rough or uneven growth surface arranged for contact with micro-biological material being cultured.
2. Apparatus as claimed in claim 1, wherein the
10 growth surface is rendered rough or uneven by surface features having a depth of 1mm or less and, preferably, a depth of 0.1-0.5mm.
3. Apparatus as claimed in claim 1, wherein said
15 growth surface is pitted.
4. Apparatus as claimed in any of claims 1-3, wherein a plurality of craters or crater like depressions are defined in the growth surface.
20
5. Apparatus as claimed in claim 3 or claim 4, wherein the growth surface is pitted or cratered to depths of 1mm or less and, preferably, to depths of 0.1-0.5mm.
25
6. Apparatus as claimed in claim 5, wherein the craters or crater like depressions measure less than 2mm, preferably less than 1mm, and more preferably less

than 0.5mm across.

7. Apparatus as claimed in any of the preceding claims, wherein the growth surface is micro-cupellated.

5

8. Apparatus as claimed in any of the preceding claims, wherein the growth surface comprises an organic polymer.

10 9. Apparatus as claimed in claim 8, wherein the polymer is coated onto a supporting substrate.

10. Apparatus as claimed in claim 8 or claim 9, wherein said polymer is a silicone rubber.

15

11. Apparatus as claimed in any of the preceding claims, wherein the growth surface forms an inner surface of a container for retaining biological material being cultured.

20

12. A method of culturing a micro-biological material, comprising culturing said material using apparatus defining a growth surface, wherein said material is in contact with the growth surface and the growth surface
25 is uneven or rough.

13. A method as claimed in claim 12, wherein the apparatus comprises apparatus as claimed in any one of

claims 1-11.

14. A method of providing a substrate with a textured surface comprising the steps of forming a mobile
5 surface studded with a granular material on the substrate, setting the mobile surface and removing the granular material to leave a textured surface on said substrate.
- 10 15. A method as claimed in claim 14, wherein the mobile surface is tacky prior to setting.
16. A method as claimed in claims 14 or 15, wherein the mobile surface is formed by softening the substrate
15 and studding the softened substrate with a granular material.
17. A method as claimed in claims 14 or 15, wherein the mobile surface is formed by coating the substrate
20 with a layer of a surface-forming material and studding said layer with a granular material.
18. A method as claimed in claims 14 or 15, wherein the mobile surface is formed by coating the substrate
25 with a layer of a surface-forming material in admixture with a granular material.
19. A method as claimed in claims 17 or 18, wherein

the surface-forming material is a silicone rubber-forming material.

20. A method as claimed in claims 17, 18 or 19,
5 wherein the substrate is treated with an adhesive prior to coating with the layer of surface-forming material.

21. A method as claimed in any one of claims 14-20 the
preceding claims, wherein the substrate is a glass or
10 an organic polymer.

22. A method as claimed in any one of claims 14-21,
wherein the granular material is soluble and is removed
by dissolution.

15

23. A method as claimed in claim 22, wherein the
granular material is sucrose, salt or potassium
nitrate.

20 24. A method as claimed in any one of claims 14-23,
wherein the textured surface is cratered or micro-
cupellated.

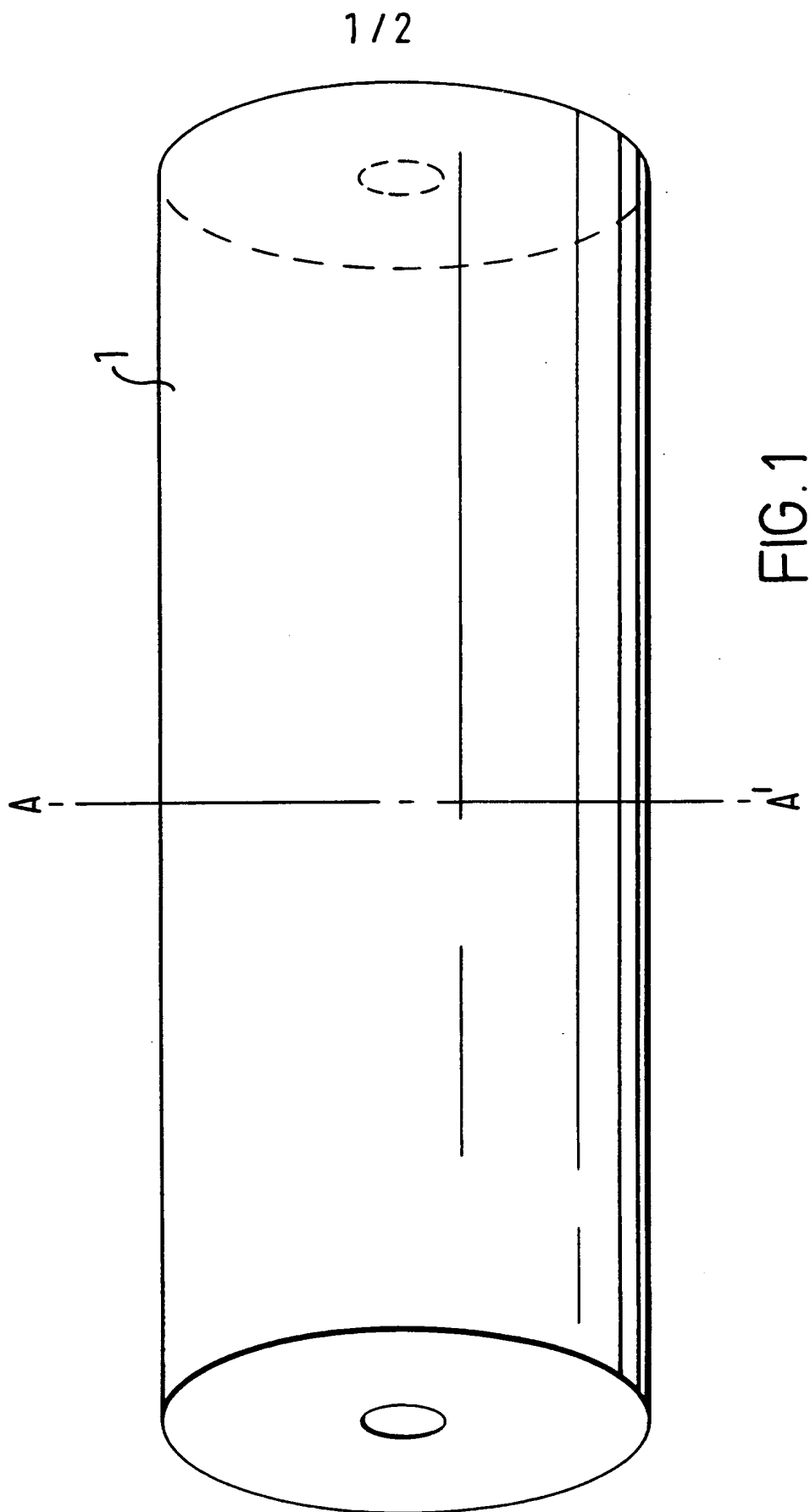
25. An article having a textured surface obtained or
25 obtainable by a method as claimed in any one of claims
14-24.

26. Apparatus as claimed in any of claims 1-11

comprising an article as claimed in claim 25.

27. A method as claimed in claim 12, wherein the apparatus comprises apparatus as claimed in claim 26.

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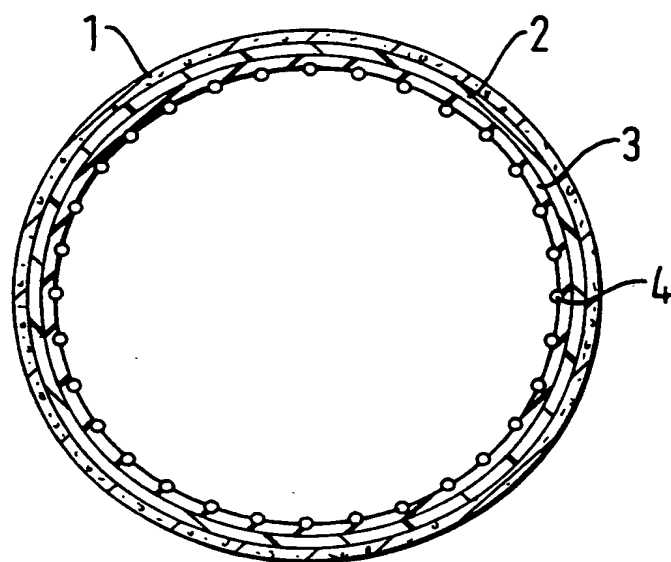


FIG. 2

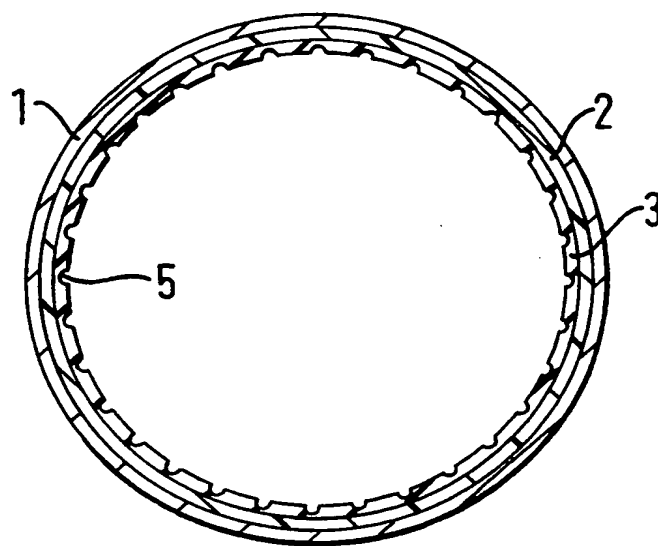


FIG. 3

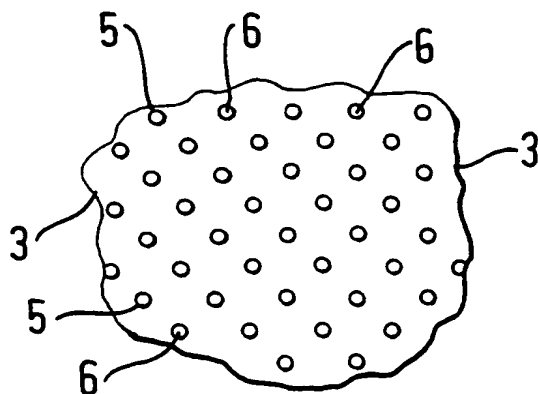


FIG. 4

INTERNATIONAL SEARCH REPORT

International Application No
PCT/GB 96/02070A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 C12M1/00 C12M3/00 C12M3/04 C08J9/26

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 C12M C08J

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

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C. DOCUMENTS CONSIDERED TO BE RELEVANT

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☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

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